

Biochemical Conversion / Lignin Utilization – Day 2

Beau Hoffman, Lisa Guay



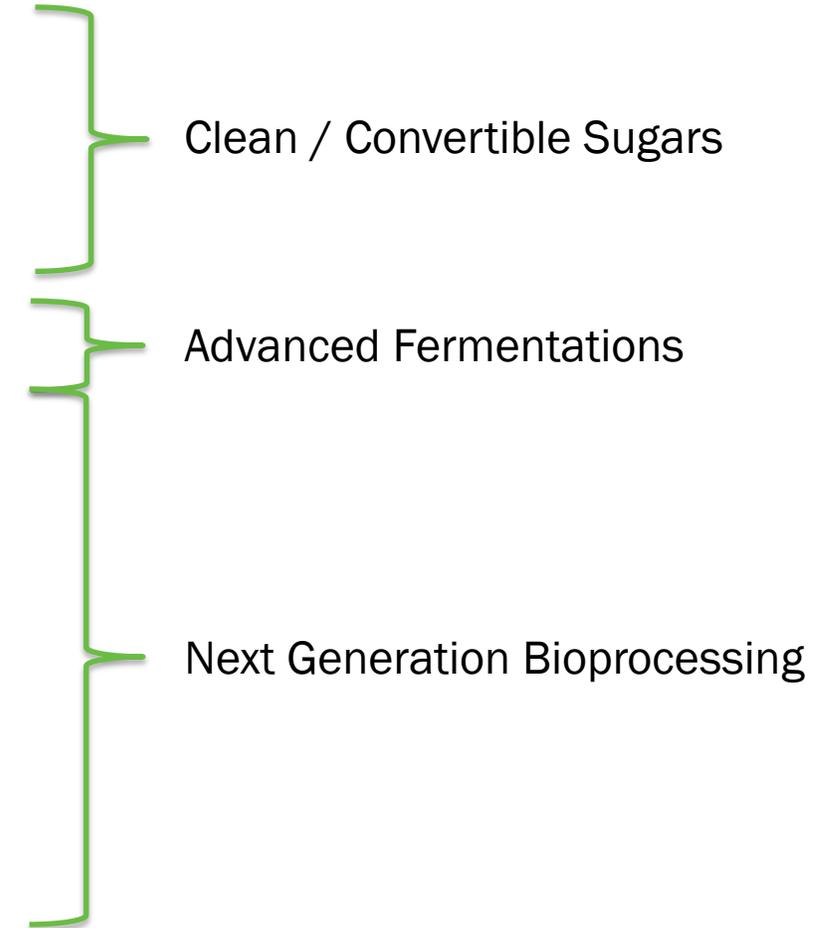
Housekeeping

Format:

- Each presentation is 20 minutes
+ 5 minutes of panel Q&A
+ 5 minutes of audience Q&A
- Ryan Lawrence will be giving time checks
(10 min, 5 min, 1 min remaining)
- Please do not take photos
(ALL presentations will be posted publicly)

Peer Review Agenda – Day 2

DAY 5 – Friday, April 7, 2023				
8:00 AM	8:30 AM	30	Registration, Breakfast	All
8:30 AM	8:45 AM	15	Technology Area Daily Intro	BETO
8:45 AM	9:15 AM	30	Continuous Enzymatic Hydrolysis Development	NREL Mike Himmel
9:15 AM	9:45 AM	30	Production of Low-Cost and Highly Fermentable Sugar from Corn Stover via Chemical-Recovery-Free Deacetylation and Mechanical Refining (CRF-DMR) Process	NREL Xiaowen Chen
9:45 AM	10:15 AM	30	Sugar is the New Crude	AVAPCO LLC Kim Nelson
10:15 AM	10:30 AM	15	Break	All
10:30 AM	11:00 AM	30	Biological Upgrading of Sugars	NREL Jeffrey Linger
11:00 AM	11:30 AM	30	Bench Scale Research and Development	NREL Nancy Dowe
11:30 AM	12:00 PM	30	Cell Free & Immobilization Technologies (CFIT) to Produce Sustainable Aviation Fuels and Other Bioproducts	NREL Yannick Bomble
12:00 PM	1:00 PM	60	Lunch	All
1:00 PM	1:30 PM	30	Towards Economical Cell-free Isobutanol Production and Cell-Free Production of Terpenoid Chemical Astaxanthin Using Crude Cofactor Lysates	Invizyne Technologies, Inc Paul Opgenorth
1:30 PM	2:00 PM	30	Engineered reversal of the β -oxidation cycle in clostridia for the synthesis of fuels and chemicals	Lanzatech (on behalf of Northwestern University) Shivani Garg
2:00 PM	2:30 PM	30	Fermentative production of Tulipalin A: a next-generation, sustainable monomer that drastically improves the Performance of pMMA	Arzeda Alex Zanghellini
2:30 PM			Adjourn	



BETO's Strategy for Today's Presentations

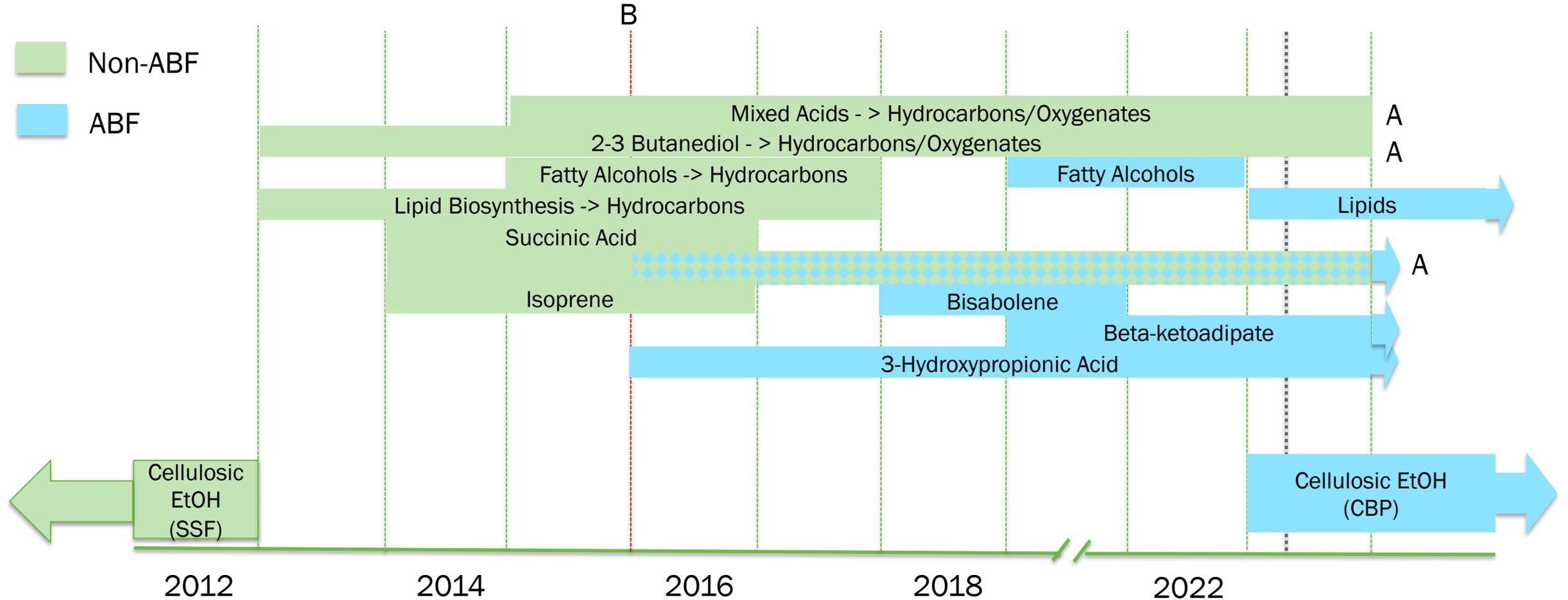
Convertible Clean Sugars

Area Goal: Demonstrate sugars with:

- >90% convertibility (titer/rate/yield) relative to dextrose (as demonstrated by multiple partners)...
- At a cost of <\$0.20/lb...
- From multiple sustainable feedstocks...
- While quantifying sustainability impacts

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8:00 AM	8:30 AM	30		Registration, Breakfast	All
8:30 AM	8:45 AM	15		Technology Area Daily Intro	BETO
8:45 AM	9:15 AM	30	2.4.1.101	Continuous Enzymatic Hydrolysis Development	NREL
9:15 AM	9:45 AM	30	2.2.3.200	Production of Low-Cost and Highly Fermentable Sugar from Corn Stover via Chemical-Recovery-Free Deacetylation and Mechanical Refining (CRF-DMR) Process	NREL
9:45 AM	10:15 AM	30	2.4.3.201	Sugar is the New Crude	AVAPCO LLC

Biochemical Pathways Through the Years



A: Undergoing merit review

B: Agile Biofoundry (ABF) Starts

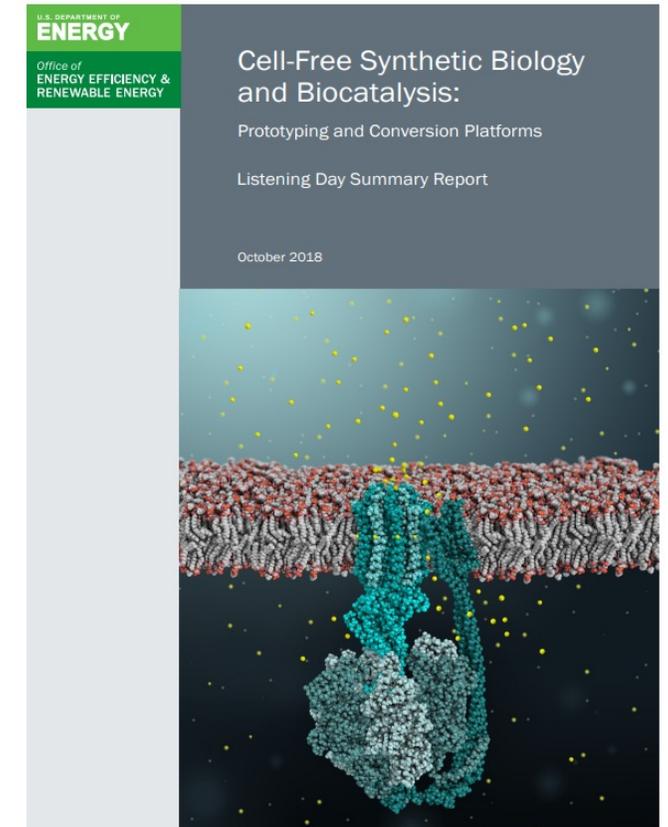
10:30 AM	11:00 AM	30	2.3.2.105	Biological Upgrading of Sugars	NREL
11:00 AM	11:30 AM	30	2.4.1.100	Bench Scale Research and Development	NREL

Next Generation Bioconversion

Area Goal: Move beyond proof of principle for the following:

- Achieving co-factor recycling/re-use
- Cell-free ‘prototyping’
- Immobilization/stabilization of enzymes

11:30 AM	12:00 PM	30	2.5.4.101	Cell Free & Immobilization Technologies (CFIT) to Produce Sustainable Aviation Fuels and Other Bioproducts	NREL
<i>12:00 PM</i>	<i>1:00 PM</i>	<i>60</i>		<i>Lunch</i>	<i>All</i>
1:00 PM	1:30 PM	30	2.5.6.203	Towards Economical Cell-free Isobutanol Production and Cell-Free Production of Terpenoid Chemical Astaxanthin Using Crude Cofactor Lysates	Invizyne Technologies, Inc
1:30 PM	2:00 PM	30	2.5.3.206	Engineered reversal of the β -oxidation cycle in clostridia for the synthesis of fuels and chemicals	Lanzatech (on behalf of Northwestern University)
2:00 PM	2:30 PM	30	2.3.4.208	Fermentative production of Tulipalin A: a next-generation, sustainable monomer that drastically improves the Performance of pMMA	Arzeda



Relevant FOAS / Funding Opportunities

Clean Sugars FOA

- Clean Cellulosic Sugars (FY21)

<\$0.20/lb
>90% convertibility demonstrated with at least 3 downstream technologies

NREL
AVAPCO

Advanced Bioprocessing

- Advanced Bioprocessing FOA (FY19)

Demonstrate cell free production
>0.5 g/L/hr for >72 hours

Invizyne

Other FOAs

- Performance Advantaged Bioproducts (FY18)
- USDA BRDI

>10% increase in bioproduct performance*

Arzeda
Northwestern U

*Relative to an appropriate benchmark technology
**Not presenting today

Recent Successes Emerging from Biochemical Conversion & Lignin Utilization Work

Genomatica and Renewable Polymers

Conversion Investments:

- 2011-2016: BC FOA supports strain development (\$5M)
- 2017-2019: Support for fermentation development and modeling in BSRD Project at NREL (~\$100k/yr)
- 2021: Subrecipient of AVAPCO Clean Sugars project

Unilever and Geno launch \$120m venture to scale alternative ingredients

BIOPOLYMERS

Published 8/17/2022 | 2 MINUTE READ

Genomatica and Aquafil Start Pre-Commercial Plant-Based Nylon 6 Production

Quantities available for evaluation in nylon applications ranging from yarns for textile and carpet to engineering thermoplastics.

lululemon partners with Genomatica on plant-based nylon

Forbes In Latest Win For The Bio-Economy, Genomatica Raises \$118 Million Led By Novo Holdings

Iowa breaks ground on first U.S. plant producing bio-BDO

May 17, 2022 / Agriculture, Biomanufacturing, State Policy / By Bio.News Staff

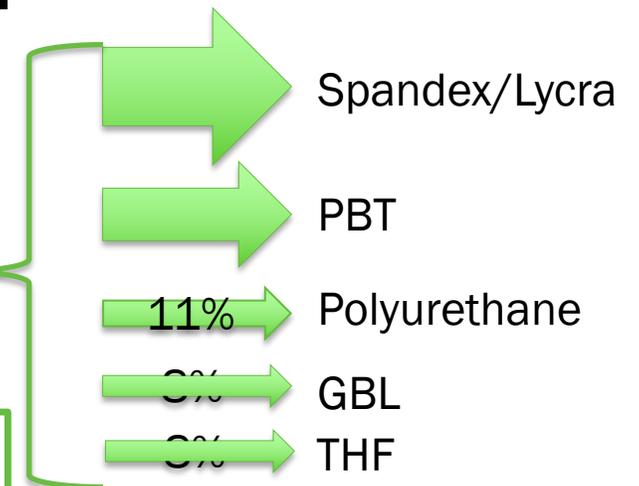
New joint venture unveils QIRA, a bio-based 1,4-butanediol (BDO) that saves up to 93 percent of greenhouse gas emissions compared to the use of conventional BDO



Fossil BDO: 4.4 kgCO₂e/kg
Bio-BDO: 0.3 kgCO₂e/kg

1,4 BDO
(2 million tons/yr)

This plant alone will result in annual emissions savings of >400,000 T CO₂e/yr

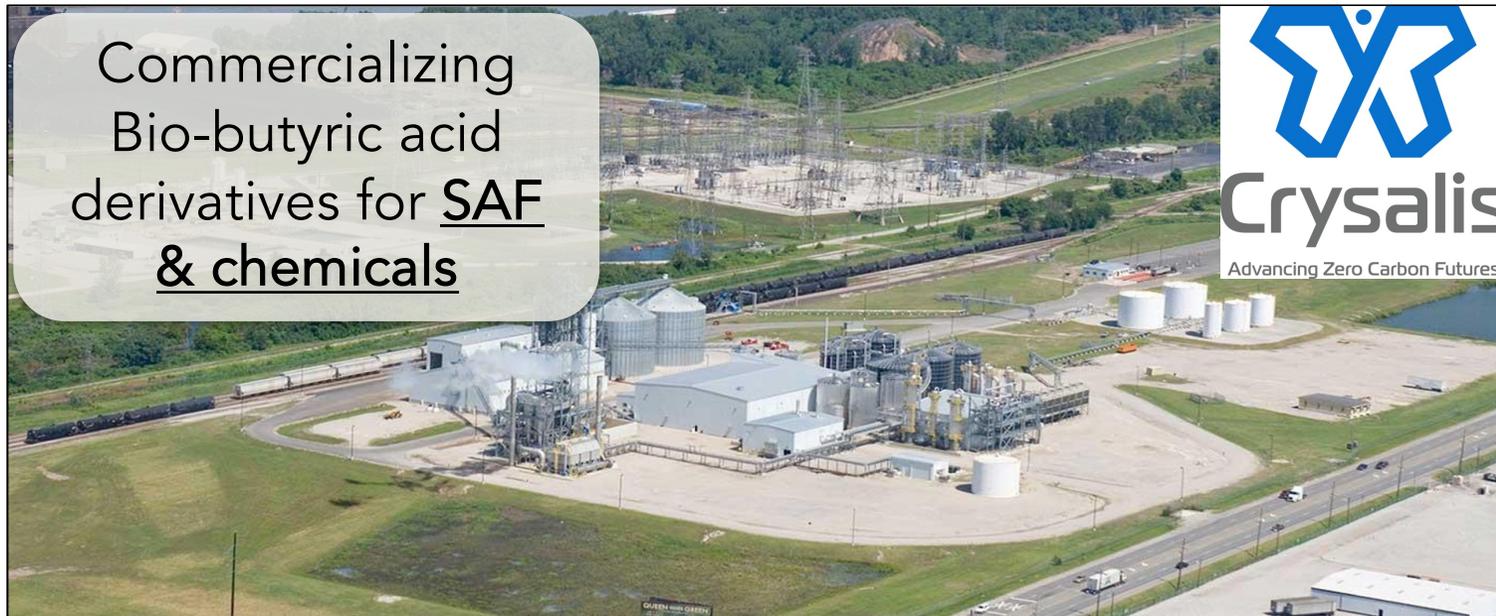


<https://bioplasticsnews.com/bio-bdo-bio-butanediol-novamont/>

NREL – Bioprincipia Partnership

Commercialization:

- NREL has developed two collaborations with industrial entities for the Technology Commercialization.



Commercializing Bio-butyric acid derivatives for SAF & chemicals

Company X

Commercializing Bio-butyric acid derivatives for human health

- Intellectual property has been licensed to both entities.
- One Cooperative Research & Development Agreement (CRADA) has been executed and another is currently being negotiated.

Corn Kernel Fiber Analytical Method Development



Renewable Fuel Standard Program

Guidance on Qualifying an Analytical Method for Determining the Cellulosic Converted Fraction of Corn Kernel Fiber

This updated 2022 guidance benefits from recent scientific advancements, including work conducted by the Department of Energy's (DOE) National Renewable Energy Laboratory (NREL), to develop a public method that addresses the analytical concerns identified in the 2019 guidance.



EPA updates guidance on corn kernel fiber ethanol production

By Erin Voegelé | September 29, 2022



ASTM Approval

June 2019

NREL/EPA Partnership Announced

Sept. 2022

EPA Issues Guidance

Sept. 2019

Gen 1.5 Workshop

Winter 2019

Method Development

Spring 2020

DOE Nat'l Lab Round Robin

Spring 2020

NIST/NREL RR Analysis

June 2020

Method/Data Sent to EPA

Cellulose (2021) 28:1989–2002
https://doi.org/10.1007/s10570-020-03652-2

ORIGINAL RESEARCH

Direct determination of cellulosic glucan content in starch-containing samples

Justin B. Shuler · Katie F. Michel · Bennett Addison · Yining Zeng · William Michener · Alexander L. Paterson · Frédéric A. Ferras · Edward J. Wolfrum

Received: 30 June 2020 / Accepted: 15 December 2020 / Published online: 15 January 2021
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Abstract A simple and highly selective analytical procedure is presented for the determination of cellulosic glucan content in samples that contain both cellulose and starch. This method eliminates the unacceptably large compounding errors of current two-measurement methods. If both starch and cellulose are present before analytical hydrolysis, both will be hydrolyzed to glucose causing bias and inaccuracy in the method. To prevent this interference, the removal of starch prior to cellulosic quantification is crucial. The method presented here is a concise in-series procedure with minimal measurements, eliminating large compounding errors. Sample preparation consists of a starch extraction employing enzymatic hydrolysis followed by a simple filtration and wash. The samples are then subjected to a two-stage acid hydrolysis. The concentration of glucose is determined by ion exchange high-performance liquid chromatography with a PS² column and a refractive index detector. The cellulosic glucan content is calculated based on the initial dry weight of the starting material. Data for the native biomass materials studied show excellent reproducibility, with coefficients of variance of 3.0% or less associated with the method. This selectivity for cellulosic glucan by the procedure was validated with several analytical techniques such as liquid chromatography coupled with mass spectrometry (LC-MS), Raman spectroscopy, and nuclear magnetic resonance.

Keywords Analytical · Carbohydrate · Cellulose · Glucose · Starch · Biofuels · Ethanol · DNP

Introduction

Conversion of starch to ethanol is an ancient technology that is still used today at industrial scale to produce renewable ethanol as fuel. Because of its history, this well-established conversion of starch, an α-(1,4)-linked glucan carbohydrate, is often referred to as a first-generation technology. Conversion of cellulose, a β-(1,4)-linked glucan carbohydrate, to ethanol, has yet to be widely commercialized but has been extensively researched (Shelton et al. 2007; Otto and McMahun 2016). This technology is referred to as a second-generation technology. In the last decade, an interesting hybrid of these two conversion

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10570-020-03652-2>.

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Springer

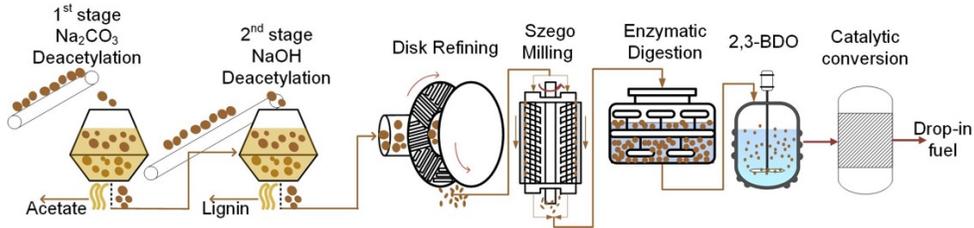
2021 Peer Review Feedback

Recommendations:

- 1) Increase use of LCA to guide research
- 2) Greater transparency in TEA methodology/assumptions
- 3) Clarify emphasis on DMR investments
- 4) Build a balance of early stage and deployment

Increase use of LCA to guide research

Examples of LCA analyses that are informing R&D priorities



	GHG* (CO ₂ e/kg)	Fossil Energy (MJ/kg)	Total Energy (MJ/kg)	Cost (\$/lb)
NaOH (100%)	2.1	28.9	32.3	0.24
Na ₂ CO ₃ (100%)	0.7	5.93	5.94	0.08

*The Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation Model (GREET)

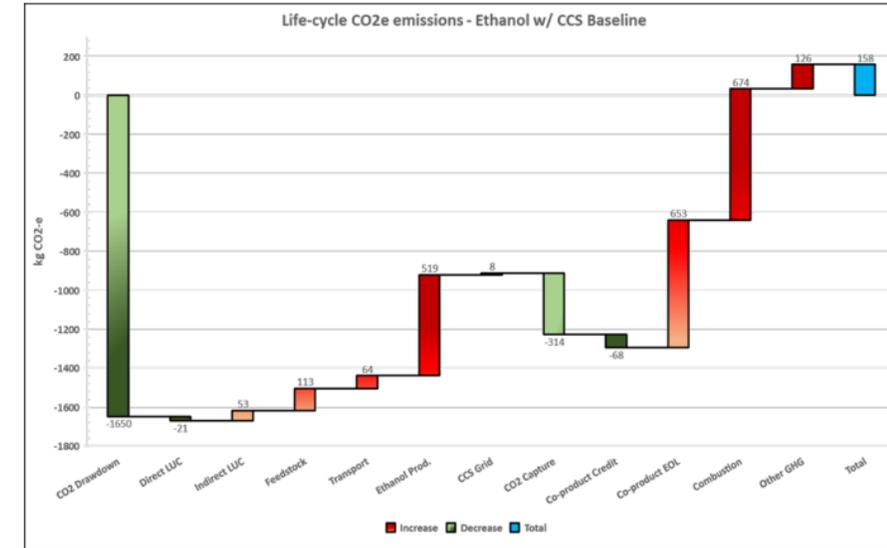
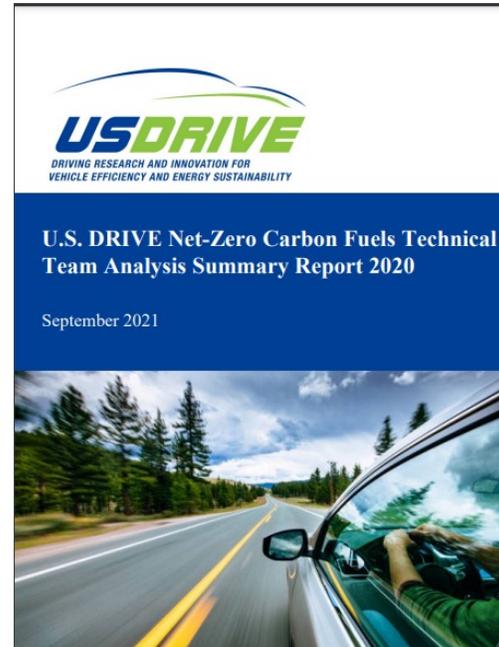


Figure 6. LCA carbon balance of fermentation with CCS only.

Topic Area 2: Affordable, Clean Cellulosic Sugars for High Yield Conversion Overview

Topic Area 2 Specific Requirements

- GHG emissions tracked in gCO₂e/lb sugars;
- Water consumption tracked in gallons water/lb sugars; and
- Carbon intensity of the process

TEA Methodology

Examples of standardized assumptions and publicly available TEA tools

Biorefinery Analysis Process Models



THIS PAGE IS TO DISTRIBUTE THIS INFORMATION TO THE PUBLIC
To save files to local computer, right click on link and choose "Save Target As...". For Aspen Plus BKP files, replace ".tiff" file name extension with ".bkp".

NOTICE: All information on this page is subject to a disclaimer.

Last Updated: November 2018

Algae Production via Open Pond Cultivation: NREL Algae Farm Model (Excel TEA Tool)
Contacts: Ryan Davis and Jennifer Clippinger

2016 Algae Farm Design Report of Davis et al. [PDF]

- Excel Spreadsheet

NREL 2017 Biochemical Sugar Model

Contacts: Ling Tao and Ryan Davis

- BKP File (Built in Aspen Plus V7.2)
- Excel Spreadsheet
- Readme Summary Sheet

Process Design for Biochemical Conversion of Biomass to Ethanol (2002 and 2011 Design Reports)

Contacts: Ling Tao and Ryan Davis

2011 Design Report of Humbird et al. [PDF]

DW1102A -- Files supporting the 2011 Design Report

Tables in the spreadsheet may differ slightly from those in the report due to small errors corrected after publication.

- BKP File (Requires Aspen Plus V7.2; does not require NREL databanks or Fortran compiler)
- Excel Spreadsheet with Macros (Requires Excel 2007 or later)

DW1107A -- Direct Port of DW1102A to Aspen Plus V7.3

- BKP File (Requires Aspen Plus V7.3)
- Excel Spreadsheet with Macros (Requires Excel 2007 or later)

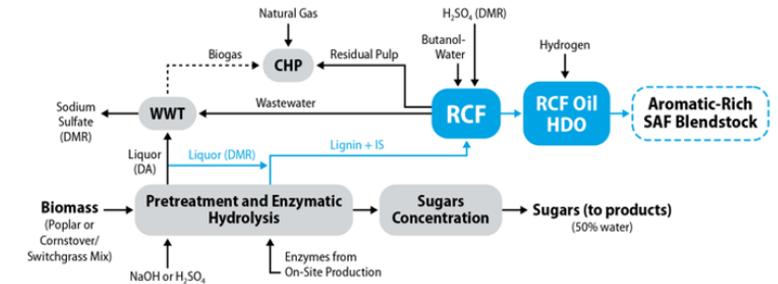
Biorefinery Analysis Process Models | NREL

Topic Area 2: Affordable, Clean Cellulosic Sugars for High Yield Conversion Overview

Topic Area 2 Specific Requirements

- Provide a techno-economic analysis to calculate the minimum sugar selling price including:
 - Cost of enzyme production and/or purchase
 - Capital and operating costs to produce monomeric sugars
 - Assuming a delivered feedstock cost of \$86/ton

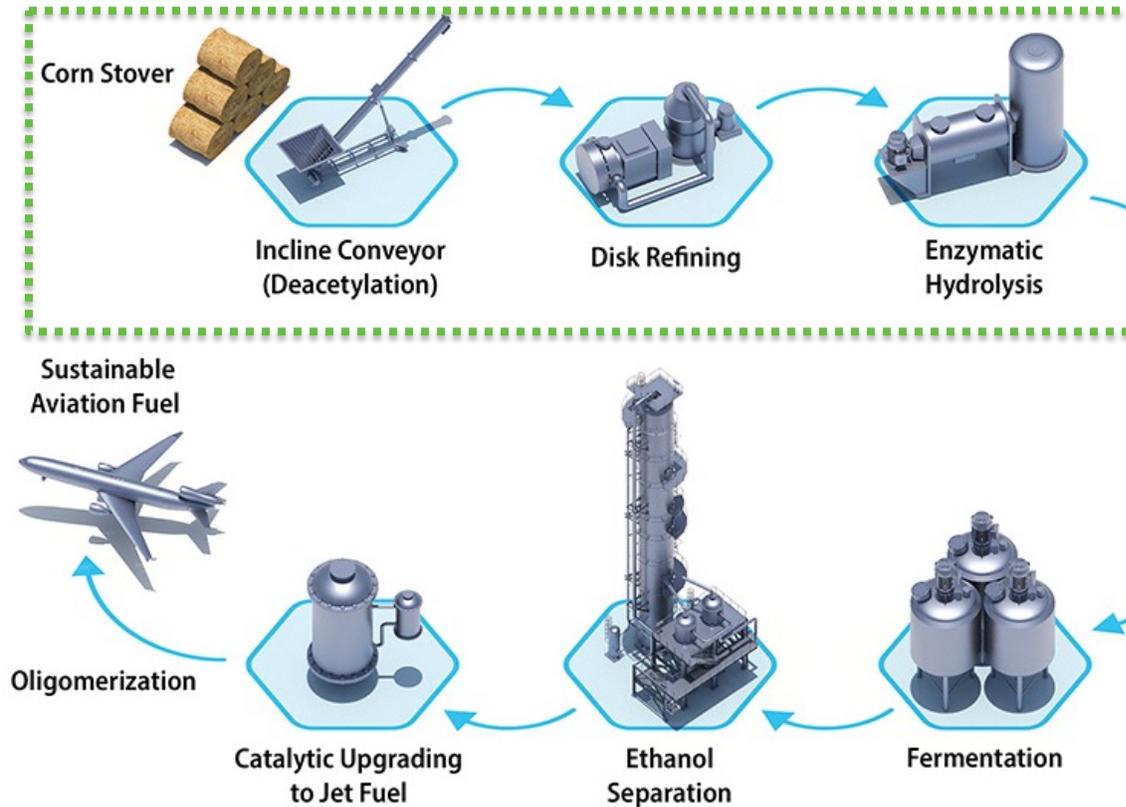
Increased emphasis on "smaller system boundary" TEA



Case	Feedstock	Pretreatment and enzymatic hydrolysis	Minimum sugars selling price (\$/kg)	GHG emissions ¹ (gCO _{2e} /MJ SAF)	GHG emission reduction
1	Poplar	DAP-EH	0.33	19	77%
2	Corn stover	DAP-EH	0.37	16	81%
3	Poplar	DMR-EH	0.53	35	59%
4	Corn stover	DMR-EH	0.60	39	54%

Why the Emphasis on DMR?

Deacetylation and Mechanical Refining (DMR)



electrek

Southwest Airlines is investing in a sustainable aviation fuel pilot program

Michelle Lewis | Jun 3 2022 - 3:02 pm PT | 0 Comments

DALLAS INNOVATES

Southwest Airlines Invests in Startup That's Turning Corn Waste into Jet Fuel

As part of a Department of Energy-backed effort, Dallas-based Southwest Airlines is investing in SAFFIRE Renewables, a new startup that aims to turn corn waste into sustainable aviation fuel.

BIOMASS MAGAZINE

Biden administration takes action to expand SAF production, use

By Erin Voegelé | September 09, 2021

BETO investments in the Pretreatment and Process Hydrolysis and Low-Temperature Advanced Deconstruction Projects 2014-2022

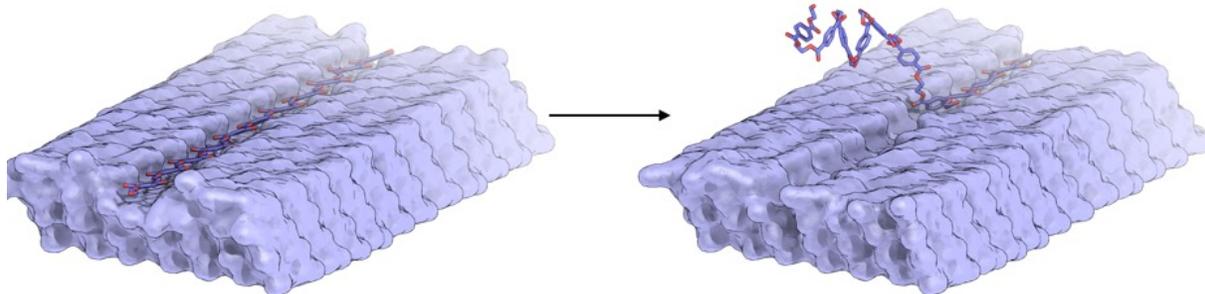


Ensure a Balance of Early and Applied TRL Work

“Therefore, this should not become an either/or for fundamental research versus applied science and large-scale demonstrations. The most powerful combination of activities for BETO would be the retention of the strong scientific program that BETO has built while advocating for new projects to support what appears to be a strong administration interest in large-scale demonstration.”

-2021 Biochemical Conversion and Lignin Utilization Panel

Analysis/Crosscutting Activities



Polymer chain in crystal surface

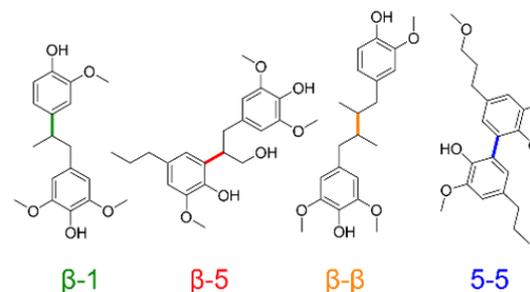
Polymer chain extracted from surface

Molecular dynamics based procedure to compute the free energy to decrystallize a single chain.

Decrystallization is the first step in many depolymerization processes, including biological routes (e.g. PETase).

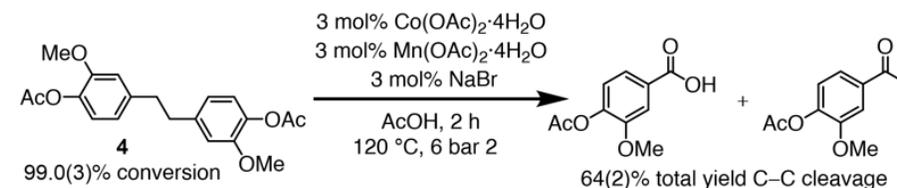
Examples of fundamental R&D that are critical for applied R&D projects:

Lignin Utilization



Exemplary C-C bonds in lignin that catalytic approaches must cleave

Exemplary C-C bonds in lignin that catalytic approaches must cleave



Gu, Palumbo, [Bleem et al.](#) in review

Questions

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